Reprinted from the Veterinary Record, May 21st, 1927.

A Consideration of some Diseases of Animals—The Laboratory and Field Worker.*

By Major T. Dalling, M.R.C.V.S., Wellcome Research Laboratories, Beckenham.

The following are some of the points included in the investigation of animal diseases—a general study of the disease as it occurs naturally in the field ; a detailed study of naturally-occurring cases, preferably in the field (under certain circumstances it may be necessary to carry out this study on animals transported to a laboratory, especially if the course of the disease is prolonged) ; a detailed macroscopic and microscopic consideration of affected tissues, including bacteriological and similar types of examinations in an attempt to name the cause. The investigation from this point may vary considerably, depending largely on the findings. As the ultimate object of research into disease is the establishment of prophylaxis and curative treatment, it naturally follows that if definite bacteriological or similar evidence is forthcoming as to etiology, attempts will be made to evolve biological products which can ultimately be used in the field. The efficacy of such products must first be established in the laboratory on laboratory experimental animals if such are susceptible, then on small numbers of the animals naturally attacked, and finally one or more extensive field trials must take place in which an adequate number of untreated animals is exposed to the natural disease under exactly similar conditions to those treated. The issue from such an experiment would decide whether the product evolved could be adopted for general use in all parts of the country. If, during the course of the investigation, the cause of the disease is shown definitely to be non-parasitic in character, the above does not apply and a further study of preventive and curative methods must be undertaken. The following remarks apply to diseases of a parasitic nature.

The laboratory and the field worker play equally important parts in investigations. To a large extent the veterinary practitioner is the field worker, and we cannot emphasise too strongly the part he can, and often does, play, and the very great help he can be to the laboratory worker. The further we study disease problems the more we must agree that in cases where the veterinary practitioner plays his part well, the earlier can results be got and the more reliable are the results of tests carried out. There are times when the laboratory worker must become

*Presented at the Annual Meeting of the North Wales Division, Bangor, February 25th, 1927.

his own field worker, for till very recently veterinary practitioners apparently took little interest in the diseases of some animals, *e.g.*, poultry, sheep, pigs, etc., but every year sees a larger number taking up the treatment of diseases of such animals and becoming of real value in further investigations.

The early and the final stage of investigations should be mainly in the hands of the field worker. Granted that the naming of the cause and pathological changes and the preparation of products for field use must be the work of the laboratory worker entirely, the field worker's share covers practically everything else, from the diagnosing of cases, the ensuring of a constant supply of investigation material, the obtaining of facilities to carry out field experiments and trials in biological products, to the final testing of preventive and curative measures on an extensive scale.

During the last three years of investigation into diseases of animals, we have tried under practically every condition to have a veterinary practitioner to play the part of the field worker. In a few cases this was impossible, either owing to the non-existence of a practitioner in the district in which the disease under study largely existed, or to the practitioner having little interest in the condition. This refers mostly to poultry investigations, yet we look forward to the very near future when poultry diseases will be included in the daily routine of the practitioner, a position in the creating of which recent and future research will, we believe, play a big part.

The diseases we wish to consider are *Blackleg in Cattle*, *Leptospiral Jaundice in Dogs* and *Bacillary White Diarrhœa in Poultry*, because they illustrate some of the points already mentioned, and some of the work affords examples of the results of collaboration between the laboratory and the field worker. It is mostly with the etiology and treatment that we propose to deal.

Blackleg in Cattle.

From 1887, when Roux investigated blackleg in cattle, till very recently, *B. chauvoei* was generally regarded as the sole cause of the disease. Even to-day there are some who incline to this view and hold that any other organisms that may be isolated from lesions of blackleg are either extraneous contaminators or are organisms which have caused a pathological condition very similar to blackleg, but still not true blackleg. The organism, other than *B. chauvoei* most commonly associated with blackleg lesions is *Vibrion septique*, which formerly was said to cause malignant œdema. Weinberg (1) in an article published lately, produces a table illustrating the finding of thirteen groups of Continental workers who examined material from 487 cases of blackleg. In 223 cases *Vibrion septique* was isolated, sometimes alone and sometimes in

association with *B. chauvoei*. Workers in this country have not had such an experience, though *Vibrion septique* has been isolated in our laboratory quite recently from a case of blackleg ; the organism was not associated with *B. chauvoei*. Bearing in mind the facts that outbreaks of blackleg, no matter what is the infecting organism, arise from wound infection with the organism which may be harboured in the soil, that the anærobic group of organisms has some members pathogenic for cattle, as seen by experimental inoculation, and that *Vibrion septique* is highly pathogenic to cattle, is more or less ubiquitous in nature and when inoculated experimentally produces lesions indistinguishable from those produced by *B. chauvoei*, it seems fairly certain that under natural conditions *Vibrion septique* will invade the animal body *via* wounds and produce clinical blackleg. The evidence in this country of *Vibrion septique* infection in clinical blackleg is not yet complete. We feel that many more outbreaks should be studied in this country to establish once and for all if organisms, other than *B. chauvoei*, may produce natural clinical blackleg. The ease with which contaminant organisms may be included in samples of muscle taken for such examinations must be borne in mind, both by the field and the laboratory worker, as well as the difficulty experienced in growing, isolating and identifying *B. chauvoei* compared with some of the other pathogenic anærobes, and the possibility therefore that it may be missed in contaminated material and a false position of importance allotted to the contaminants.

Because of the belief that *Vibrion septique* may play a not unimportant part in the causation of blackleg, some recent vaccines to protect against this organism and *B. chauvoei* have been made in this country and on the Continent. We have shown on several occasions that a *Vibrion septique* toxin-antitoxin mixture can be made, which, after two injections, will produce a high degree of immunity in guinea-pigs and in sheep ; a dose of toxin or culture lethal to unprotected animals is tolerated with ease by those protected by the mixture. It has also been shown that a moderate degree of immunity results after one injection of such a mixture. Further, it has been shown by many, including ourselves, that complete immunity may be found in guinea-pigs and sheep following a single dose of *B. chauvoei* filtrate. Equal parts of *Vibrion septique* toxin-antitoxin mixture and *B. chauvoei* filtrate are used to make the vaccine referred to. Field trials of this vaccine have been satisfactory, but all field workers who have carried out the tests are not in favour of two doses being given, because of the expense incurred in the inoculations. At the present time there is no evidence to show that any preparation made by using *Vibrion septique* will, after one injection, produce a high degree of immunity in the field, though McEwen (2) has recorded results of some work on the subject which show promise

that such a product may be evolved. One dose of *B. chauvoei* filtrate, however, will completely immunise. If it is desirable to use the double vaccine and only one dose can be administered, complete protection against *B. chauvoei*, and some protection against *Vibrion septique* will result. Some protection is better than none at all; hence, even though only one dose may be injected, it would appear that the double vaccine is indicated in the light of our present day knowledge.

For the treatment of cattle affected with blackleg, doubtless every practitioner has his own pet remedy. It may be of interest to know that some practitioners rely on the injection of anti-*B. chauvoei* serum. Such a serum can be produced and some laboratories issue a serum which, when tested in the laboratory, has the power to check infection by *B. chauvoei*.

Lately, it was necessary, for some laboratory work, to use an anti-*B. chauvoei* serum and we made some in a cow by injecting *B. chauvoei* filtrate.

(Table 1 shows that the serum was a true anti-bacterial *B. chauvoei* serum.)

TABLE 1.

Blackleg Serum—Made in Cow.

Guinea-pigs received serum two days before culture.

Serum.	Culture.	Result.
5 c.c.	0·2 c.c.	L.
2·5 c.c.	0·2 c.c.	L.
1·0 c.c.	0·2 c.c.	L.
1·0 c.c.	0·1 c.c.	L.
Normal Cow Serum	0·1 c.c.	D.
No Serum	0·1 c.c.	D.
	0·1 c.c.	D.

L =Lived. D =Died.

High grade *Vibrion septique* antitoxin can also be produced.

CANINE LEPTOSPIRAL JAUNDICE.

Okell, Dalling and Pugh (3) intimated that cases of canine leptospiral jaundice had been studied and *Leptospira icterohæmorrhagica* isolated. We believe this parasite to be the cause of outbreaks of canine jaundice or "yellows"—a disease which occurs as an enzootic in many kennels. It must be pointed out here that the name canine leptospiral jaundice or "yellows" does not include sporadic cases of jaundice which the practitioner frequently sees in dogs, but refers to that highly-fatal infectious disease during the course of which many or all of the dogs in a kennel may be affected. The disease has been reported to us from all over England and Scotland and is most commonly found affecting young dogs in sporting kennels.

The parasite is found normally in the kidneys of male wild rats, and would appear to be harboured there without interfering with the general health of the animal. It is

voided in the urine and may thus soil and contaminate food, litter, etc., to which the rats have access. Houston (4) has demonstrated the presence of leptospira in well water an many occasions, and in some cases it would appear that rats could not have had access to the water. Okell has been able, working with water samples from the same wells, to show that one sample contained leptospira which were pathogenic for tame rats and guinea-pigs. Many leptospiræ were seen in the other samples, but on experiment they proved non-virulent. Buchanan (5) working on slime obtained from parts of coal mines in Scotland was able to demonstrate living, virulent leptospiræ which were believed to have caused cases of Weil's Disease in some miners working in the mines. The slime was in such a position that rats could not have had access to it. It would appear, then, that though rats are the important vectors of the disease in this country, other sources do exist, though possibly in the case of dog infections they are of little consequence.

It is a very simple process to infect dogs with virulent parasites in the laboratory. Whereas our original method was to injeet a suspension of mashed up guinea-pig liver or cultures intraperitoneally, we have shown that infection can be brought about by feeding the organism in the food and by scratching the dog's skin and laying infected material on the scratched area. In all our recent infection experiments we have used the scratch method with complete success. We can, therefore, now definitely state that dogs can be infected by picking up infected material in food or water and through skin abrasions on any part of the body, including the pads of the feet.

Treatment of leptospiral jaundice is more successful from a prophylactic than a curative aspect. Serum and vaccine have been prepared and have been shown to be effective.

Serum is made in horses by repeated injections of killed or attenuated organisms. In the laboratory it has been shown to protect guinea-pigs and dogs when administered before, at the same time as, or following the living parasites. In practice its value as a curative agent is very problematical because of the rare occasions on which it can be injected early in the course of the disease. Experiments already reported show that icteric symptoms develop about 4—5 days after infection has taken place, and that when gross infection is present, serum can do little to save the animal's life after jaundice is established. Its value is its ability to produce a passive immunity of about three weeks' duration, and therefore its chief use is in preventing the spread of the disease in an infected kennel. Inoculations done on all the dogs in a kennel in which one or more cases have been diagnosed will prevent their infection and allow time for the removal of the infecting agent. We have much proof on this point. Although we have shown experimentally that the appearance of icterus in

the very severe infections produced in laboratory work marks the end of the usefulness of the serum, many veterinary surgeons have had good results from its use. even at this stage of the disease. The results reported to us vary, but there is some chance that in the field, even in dogs showing marked icterus, recovery may occur after injection of serum.

Vaccine treatment has given good results in the laboratory and in the field. Its use should be confined to dogs in kennels in which periodic outbreaks of jaundice occur, and in which no actual cases have occurred for some time prior to its use. During the period immediately following injection the dog may be more susceptible to the action of the parasite, hence care should be taken that no cases of disease are present at the time of vaccine injection. Our former experiments have been carried out with vaccine made from guinea-pig liver; lately we have succeeded in culturing leptospira on artificial media in bulk and have experimented on dogs with a vaccine made from such cultures. Complete protection to tests of virulent material results, following two inoculations of treated culture in doses of even 0·1 c.c.

TABLE II.

Leptospira Vaccine—Made from Cultures.

Dogs 69—71	0·1 c.c.	7/8/26 and	20/8/26
,, 72—74	1·0 c.c.	,, ,,	,,
,, 75—77	0·1 c.c.	20/8/26 and	27/8/26
,, 78—80	1·0 c.c.	,, ,,	,,

All dogs tested 10/9/26.
Dogs 69—80—All lived.
,, 81—85 (which had received no vaccine)—All died from jaundice.

BACILLARY WHITE DIARRHŒA IN FOWLS.

We refer to this disease because of its recent increasing prevalence and because we feel that the veterinary practitioner must be in a position to advise on its prevention and cure. The rate of spread of B.W.D. all over the country must be expected from the numbers of all kinds of fowls being bred from to-day without adequate supervision by authorities on disease of poultry. The annual losses in chicks from B.W.D. are enormous and the present hatching season is even worse than the last, from reports already to hand.

B.W.D. is fatal to young chicks only, but adult fowls may harbour the infecting agent (*B. pullorum*) while appearing perfectly normal and healthy. Such fowls are termed "carriers," and they are responsible for the perpetuation of the disease from year to year. Rettger and others have worked out the cycle of infection. We have confirmed this work in this country. Chicks may be hatched out in an infected state, or, in some cases, may be

infected during the first few days of life by picking up contaminated excreta, etc. The mortality among such chicks varies greatly, depending on the virulence of the infecting agent. Practically every chick in the hatch may be wiped out, or, if the infection is light, the death rate may be extremely low. Survivors in the infected hatch often become " carriers," *B. pullorum* being located in the ovary and infecting many of the eggs laid when the chick reaches maturity, so that the offspring of such hens in their time produce chicks which die of B.W.D., infect other chicks or become " carriers."

We have studied methods for the prevention of the disease, realising very early in our work that curative methods were unpractical. Two points have interested us specially : prevention of spread among chicks and diagnosis of the carrier fowl. Experiments now in progress indicate that where there is reason to suspect B.W.D. from hatching eggs, the dividing of the young chicks into small units tends to prevent its spread. This is in keeping with epidemiological expectations and only concerns the chicks hatched from non-infected eggs in contact immediately after hatching with infected chicks. There is little hope for the chick from infected eggs ; our aim must be to prevent the breeding from fowls capable of producing infected eggs, and, if this is not practical, to prevent the spread from chick to chick.

Small lots of an anti-serum have been made by injecting hens, rabbits, sheep and goats with *B. pullorum*. Laboratory tests with this serum have shown that this disease can be prevented in healthy chicks provided the serum is injected at the same time as, or prior to, the feeding or injecting of the causal organisms. Field trials have been done on a limited scale, but in an experiment in which over 2,000 day-old chicks, bred from hens in which about 50 per cent. were shown to be " carriers," were injected, our conclusions were that serum does prevent infection from chick to chick, but does little to help the " egg-infected " chick. Further experiments are now in progress.

Diagnosis of the " carrier " hen can be made by carrying out agglutination tests or by the results from the intradermal wattle test. Both tests have been described by American workers. We have had occasion to study both methods and have concluded that both may be useful. It is necessary to repeat tests at intervals on all stock passed clean by either test, but we believe B.W.D. can be cleared entirely from any flock by adequate testing of the breeding fowls. The cockerel should be included in all tests done.

We hope to publish an article soon on results of our work on B.W.D.

We do not feel it necessary to point in detail to the relationship of the laboratory and field workers in the above work, but we do want to emphasise that much has been made possible by their close collaboration.

References.

(1) Weinberg and Ginsbourg.—*Bull. de l' Inst. Pasteur.* Vol. XXIV. No. 20.
(2) M'Ewen, A. D.—*Journ. Comp. Path. and Therap.* Vol. XXXIX. Part 4.
(3) Okell, Dalling and Pugh.— *Veterinary Journal.* Vol. 81. No. 1.
(4) Houston, A.—Metropolitan Water Board 20th Annual Report, 1925-26.
(5) Buchanan, G.—*British Medical Journ.*, 1924. Journ. II. p. 990.

Discussion.

In opening the discussion, Mr. Montgomerie expressed his particular appreciation of the paper which Mr. Dalling had presented that afternoon. Mr. Dalling was ready to make the long journey from London and had read one of the most interesting and thoughtful papers to which it had ever been their pleasure to listen.

Proceeding, Mr. Montgomerie said, "We have had the normal course of an investigation outlined and the field worker has been assigned his due and proper place in the scheme. It might well be emphasised that the field worker, especially the practitioner, should come first on the scene. He has the opportunity to come in contact with conditions which require investigation and should be the person who draws the attention of the laboratory worker to the problem. Frequently the call comes from another source—a section of the agricultural community, a breed society or other such organisation.

"Occasionally the laboratory worker may appear to invade the field of his colleague, for the careful large scale test of a product, with the necessary controls, is often a time-consuming job which the practitioner cannot undertake. An example of this is to be found in the trial of vaccines and sera prepared for the prevention of lamb dysentery. Yet such products reach a more or less commercial stage when the observations of the practitioner-field-worker, however general they may be, are appreciated by the laboratory worker.

"The suggestion that, in this country as well as abroad, blackleg of cattle may frequently be caused by an organism other than *B. chauvoei* may appear rather disturbing. Vaccination against blackleg in cattle is one of the most successful preventative inoculations we possess—one which has brought our profession much credit. We have believed that these vaccines were made from material containing *B. chauvoei* or its products. There is no cross immunity between *B. chauvoei* and *Vibrion septique*, yet we know these vaccines are highly successful. Remembering that anærobes have only been carefully isolated and studied in the post-war years we are entitled to wonder

if these vaccines are made from pure cultures. Yet we have been told that a double inoculation is necessary to protect against *Vibrion septique* and we know that some of the blackleg preventatives are used as a single inoculation.

"I think text-books tell us that the *B. chauvoei* generally gains entrance to the animal body through infection of a wound in the alimentary canal, and, if I remember correctly, some experiments were conducted which supported this idea. Can Mr. Dalling say if the feeding of *Vibrion septique* under conditions likely to cause injury to the alimentary canal will produce typical blackleg? That on subcutaneous injection this organism does give rise to a blackleg lesion is hardly proof, unless, of course, you accept external wound infection as the usual route.

"While two injections of vaccine made by most methods are necessary to give protection against *Vibrion septique*, McEwen has recently introduced a mode of preparing the vaccine which, by a single injection, gives protection. This piece of work is of much interest and significance. I look forward to reading the result of an extensive field trial of formalised vaccine prepared as McEwen suggests.

"I have not had any experience of anti-*B. chauvoei*, and I wonder it if is widely employed. Do practitioners see a sufficient number of blackleg cases alive to make it worth while stocking this serum? If this serum is effective does its action not favour the opinion that *Vibrion septique* is much less a cause of blackleg in this country than the figures of continental workers might suggest?

"In North Wales we hear of many losses among sheep characterised by sudden death and variously named (*e.g.*, strike), according to the locality, time of year, etc. There are those which appear to be true braxy, but I would be glad if Mr. Dalling could tell us which organism causes those deaths in Spring amongst ewes and forward lambs which we meet, or hear about.

"In offering canine leptospiral jaundice for your consideration we are presented with a splendid example of the result of good team work. One can readily picture Pugh bringing this disease, together with a wealth of clinical *data* and material, to the notice of the laboratory workers. The investigation of 'yellows' was so admirably and skilfully conducted by these three workers that it must be regarded as one of the premier achievements of recent years.

"So far, bacillary white diarrhœa has attracted little attention within North Wales, for it is only recently that poultry keeping has extended beyond the 'barn-door' type. Mr. Dalling has said that the disease is fatal to young chicks only. I would ask if he has not met the condition as an acute infection of adult birds. Is there any spread of the disease from adult birds directly to other stock, and what is the significance of the test on the

cockerel? May he transmit the disease? I am interested to hear that the intradermic method of testing may be developed, for I believe that many practitioners will prefer the latter. In using it they can read the results themselves and, without delay, yet with great certainty, they can pick out the reactors from the breeding stock.

"I am sure that each of us will wish to discuss this paper and add our quota of thanks to Mr. Dalling."

Others present having contributed to the discussion, Mr. Dalling, in reply, thanked those present for the welcome he had been given and said that the lively discussion that had taken place had amply repaid him for any trouble to which he had gone in preparing the paper and in coming to Bangor to address the Division.

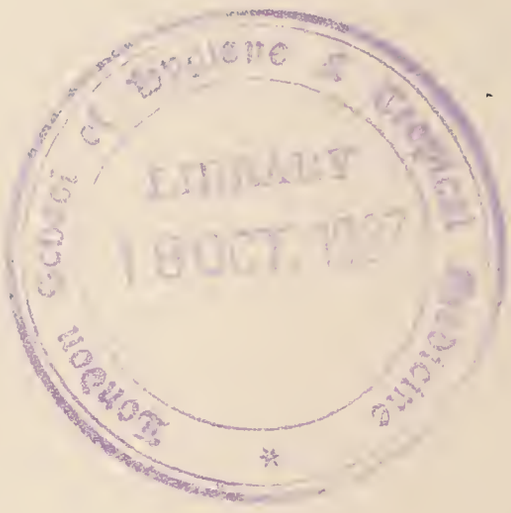
The question of the cause and prevention of blackleg in cattle had been dealt with by Mr. Montgomerie and most of the other speakers. He quite agreed that the disease as it occurred in Britain would appear to be caused mainly by *B. chauvoei*. The fact that histological products made from *B. chauvoei* afforded such protection in the field would amply justify one in making such a deduction. There were, however, instances in which vaccination with such products had apparently yielded poor results. One was cited that day by Mr. Roberts, and occasionally in blackleg districts there were farms on which the disease was not fully protected against by *B. chauvoei* biological products. It would be of considerable scientific interest to investigate the cause of the so-called blackleg on such farms. Another point of which sight must not be lost was the position even to-day in the isolation and purity of anærobes, and therefore the question of the true character of agents used in the immunisation against blackleg. One inoculation of products made from *Vibrion septique* resulted in a certain number of injected animals being immunised. As far as was known, the feeding of cattle and sheep with *B. chauvoei* and *Vibrion septique* cultures had not produced any symptoms of recognisable disease. Research had still to show the necessary conditions of the various body systems for infection to take place in most diseases. From the descriptions given by Mr. Montgomerie, it was probable that some anærobic infection was prevalent in the lambs dying of so-called 'strike.' It had still to be shown that *B. chauvoei* infections occurred in sheep in this country under natural conditions. Protection experiments could be devised to show if an anærobic infection was present in the lambs, and, if so, which was the infecting agent. Investigations were at present in progress on diseases affecting ewes before and after lambing in various parts of the country.

Leptospiral jaundice was a disease quite apart from distemper, though without doubt it would attack dogs suffering from or recovering from distemper. It was caused by a Leptospira, while recent work had confirmed

the view that distemper was caused by an ultravisible virus.

It was evident from the discussion that B.W.D. did not exist to a great extent in North Wales. Poultry keepers there must be in a much happier position than those in the South of England. Infections did occur in adult stock and the causal agent was presumably the same as that infecting chicks. The incidence of the disease in adults was small compared with chicks, though the literature would show that "carrier" hens might, when penned up with clean birds, cause them to become reactors; recent experiments had not confirmed such work. Cockerels should always be included in tests for "carriers," as a small percentage had been shown to react.

HUDSON AND SON, PRINTERS, BIRMINGHAM AND LONDON.


London School of Hygiene & Tropical Medicine
LIBRARY
18 OCT. 1927